Improved lipids extraction / Lipids analysis by GC-MS and Flow Cytometer

Guillaume Delfau--Bonnet[a,b], Tiphaine Clément[a], Florent Allais[a], Anne-Lise Hantson[b]

[a] Chaire ABI AgroParisTech, Pomacle, France
[b] Laboratoire de Génie des Procédés Chimiques et Biochimiques, Université de Mons, Mons, Belgique
*(guillaume.delfau-bonnet@agroparitech.fr, guillaume.delfau--bonnet@umons.ac.be)

Key words: *Yeasts, Microalgae, Lipids, GC-MS, Flow Cytometer, Yeasts, Microalgae*

The production of microalgae could be huge in few years. A sustainable valorization of this biomass is necessary to prevent waste generation. The ALPO project aims at valorizing lipids produced by microalgae through biopolymers production. To be sustainable in an industrial context, the process must take in consideration the valorization of every part of the microalgal biomass i.e lipids but also secondary metabolites and co-products issued from these molecules extraction process (Microalgae Extraction Co-products, MECP).

Fermentation can be a way of valorizing the parietal sugars of microalgae that remain after lipids extraction. In particular, in the context of the ALPO project, the use of oleagineous yeasts is investigated.

The characterisation and the accessibility of the fermentable sugars are two crucial points to understand the best way to valorise the MECP. Three different microalgae species (*Nannocloropsis gaditana* and *Chlorella vulgaris* as dried biomass, *and Nannocloropsis oceanica* as wet biomass) were selected to investigate their composition after lipids extraction.

Several lipid extraction protocols were tested. They were modification of Bligh and Dyer protocol's (1). The selection of the extraction protocol was based on the mass of lipid extracted, the length of the extraction and its practicality. Only two methods validated these criteria and just one was saved for the rest of study.

In parallel, a method is developed to analyze lipids accumulation in oleagineous yeast during fermentation. This method based on flow cytometry uses fluorescent markers such as Bodipy and Nil Red to specifically detect lipids in yeasts without needing time consuming extraction. This technique will be tested on different oleagineous yeast. The comparison of lipid accumulation in the different yeasts during fermentation, in synthetic medium as well as on MECP, will allow choosing the best strain for its application to microalgae biorefining.

To the next step of the project, a method development will be necessary to analyze carbohydrates in microalgae wall.

Reference :

1. Bligh EG, Dyer WJ. 1959. A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. Can J Biochem Physiol 37:911–917.

Acknowledgements :

European Territorial Cooperation Programme Interreg between France-Wallonie-Vlaanderen

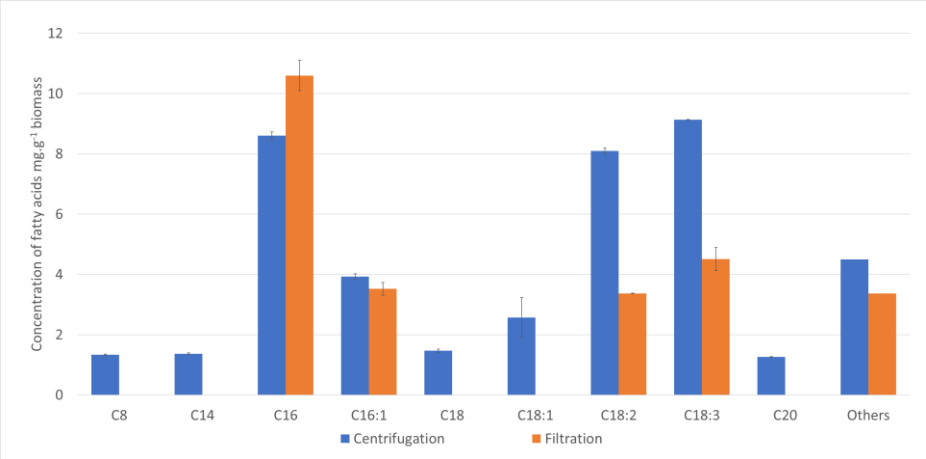

***Figure 1**: Efficiency of centrifugation and filtration methods based on Bligh and Dyer protocols for lipid extraction from Chlorella vulgaris*

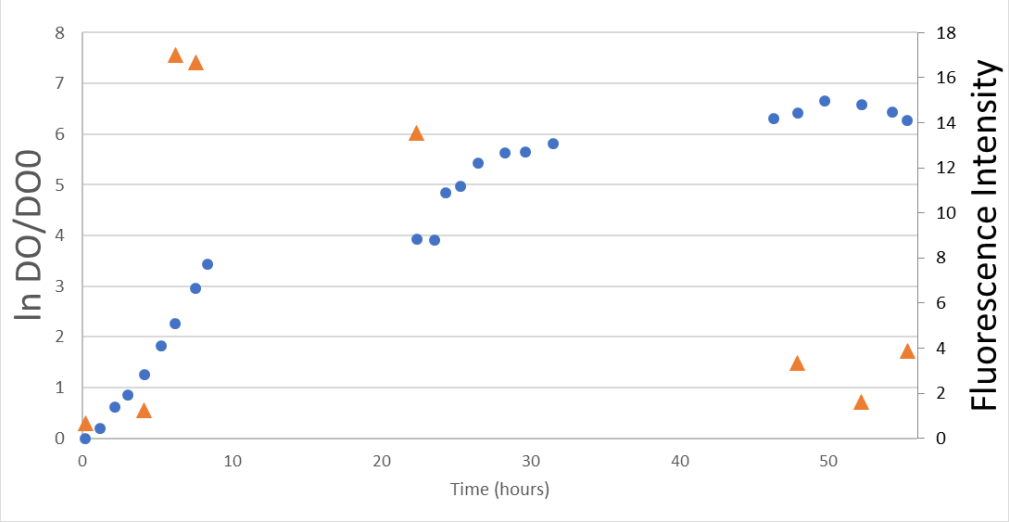

Figure 2: *Monitoring of lipids production during fermentation of Yarrowia lipolytica by flow cytometry*